



# Effect of some environmental factors on seed germination of *Eryngium caeruleum* M. Bieb. populations

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## ABSTRACT

The effects of alternating and constant temperatures and light regimes, osmotic and salt stress and pH were tested on seed germination in four populations of *Eryngium caeruleum*. Laboratory experiments revealed that the four populations exhibited different responses to alternating temperature and light conditions. Alternating temperature and photoperiod had a greater positive effect on seed germination compared to complete darkness. The optimal constant temperature within 10 °C to 15 °C for seed germination of each population was determined in a light/dark photoperiod. Seed germination severely decreased under water stress and was completely inhibited at -0.8 MPa osmotic potential. Saline stress sharply decreased germination percentage. Germination was restricted by pH lower and higher than 5 and 8, respectively. The information obtained from this study helps to fill the gap of knowledge about seed germination requirements of *E. caeruleum* and enhance our understanding of this species distribution and its potential to develop in stressful and/or new habitats.

**Keywords:** alternating and constant temperatures, germination, light, pH, salt and osmotic stress

## Introduction

The distribution of plant species among habitats is determined by a wide range of climatic and edaphic factors. Habitat heterogeneity combined with natural selection often lead to multiple, genetically distinct ecotypes within a single species (Linhart & Grant 1996). Thus, ecotypes are populations of a particular species that are evolutionally adapted to specific environmental conditions. These ecotypes, or populations that occurring in distinct habitats, vary from one another in morphological traits such as shape, size, or leaf color (Krawczyk & Krawczyk 2000), as well as traits related to seed germination.

Seed germination is considered to be the most vulnerable and crucial phase in a plants life cycle (Finch-

Savage & Leubner-Metzger 2006). Temperature is known to be one of the most influential environmental factors for seed germination (Finch-Savage & Leubner-Metzger 2006). Some plant seeds germinate at temperatures above a certain minimum threshold, whereas others need daily fluctuations in temperature above and below a specific temperature. The germination of many annuals plants increases with alternating temperatures (Bazzaz 1979). Temperature is also related to germination through altering levels of gibberellins and abscisic acid in the seed (Finch-Savage & Leubner-Metzger 2006). Salt stress and water deficit are key environmental factors that can hinder or halt seed germination. Soil pH is an edaphic factor that can influence the distribution of plants, and some plant species germinate in a wide pH range (Yazdi *et al.* 2013;

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Rezvani & Yazdi 2013), while for others it can be a limiting factor (Amini *et al.* 2015).

The genus *Eryngium* of the family Apiaceae includes about 250 species distributed throughout Eurasia, North Africa, North and South America and Australia. The genus *Eryngium* is considered to be the most species-rich genus of Apiaceae (Pimenov & Leonov 1993). Species of *Eryngium* are distributed among a wide range of environmental conditions in Iran, from semi-arid to temperate regions (Karimi 2001). *Eryngium caeruleum* is a perennial herbaceous plant that is native to northern Iran (Ghahreman 1997). This plant's life cycle begins in mid October from permanent roots in the soil and/or seed, and reaches maturity late in July (Karimi 2001). *Eryngium caeruleum* is used in folk medicinal and as a spice plant in different regions of Iran. Root, leaf, stem and inflorescence of the plant have medicinal properties. The fresh leaves of the plant have a good taste and are aromatic, and so it is used in traditional cooking (Khoshbakht *et al.* 2006; Daneshfar *et al.* 2014). Species of *Eryngium* are propagated by seed production, transplanted plantlets or by cuttings (Armitage 1993). The growth rate of *Eryngium* is very slow, so their ability to respond to habitat disruption is weak (Everett 1960). Seeds of species of Apiaceae are often morphologically or morphophysiologicaly dormant (Finch-Savage & Leubner-Metzger 2006).

Nothing has been published on the factors that affect seed germination of *E. caeruleum* and its ability to tolerate conditions of saline and drought. The aims of the present study were to: (1) determine the effects of alternating and constant temperature and light regimes, and osmotic and salt stress in different temperature regimes and pH, on seed germination of four populations of *E. caeruleum*; and (2) document how the growth conditions of the mother plant affect traits of seed germination.

## Materials and methods

### Seed source

Seeds of four populations of *Eryngium caeruleum* M. Bieb. were collected in late July 2014, from 300 naturally matured and completely senesced plants. The climatic and geographical characteristics of the region of where the populations are growing are shown (Tab. 1). Seeds were separated from chaff and stored in paper bags at 20±5 °C before starting the experiments.

**Table 1.** Geographical and weather parameters of populations growing locations.

Populations	Coordinates	Height (m)	Rainfall (mm)	Average temperature (°C)	Minimum temperature (°C)	Maximum temperature (°C)
P1	36°49'N; 53°26'E	50.02	665	16.01	-5.40	38.20
P2	36°66'N; 53°66'E	-17.2	665	16.01	-5.20	38.60
P3	36°48'N; 53°28'E	147	539.80	12.88	-5.20	38.60
P4	36°42'N; 53°36'E	1875	306.10	10.08	-15.40	35.80

### Seed germination test

Prior to the experiments, thirty seeds of *Eryngium caeruleum* were evaluated for viability by 1 % tetrazolium chloride solution (Peters 2000).

Seeds were sterilized with 1 % sodium hypochloride for 2 minutes and then washed with distilled water five times. For each experiment 30 disinfected seeds were placed on two layers of Whatman No. 1 filter paper and moistened with 6 ml distilled water or test solutions. Petri dishes were sealed with Parafilm, to inhibit water reduction, and incubated. Fluorescent lamps were used to produce a photosynthetic photon flux intensity of 150- $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Germinated seeds, as indicated by the visible protrusion of about 1 mm of the radicle, were counted after 15 days.

### Effects of alternating temperature and light regimes on seed germination

Initial seed germination of seeds of *E. caeruleum* from the study populations was evaluated in different alternating temperatures, including 20/10, 25/15, 30/10 and 35/15 °C (day/night), in a 12/12-hour light/dark (day/night) photoperiod and complete darkness. For complete darkness, Petri dishes were wrapped in two layers of aluminum foil.

### Constant temperature and light regime

Germination of seeds from the study populations was evaluated in constant temperatures, including 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C, both in a 12/12-hour light/dark (day/night) photoperiod and complete darkness. For complete darkness regime Petri dishes were covered in two layers of aluminum foil.

### Effect of osmotic potential on seed germination in different temperature regimes

The effect of osmotic stress on the germination of seeds from the study populations was evaluated in solutions of 0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1 MPa, prepared by dissolving 0, 91.6, 129.5, 183.1, 224.2, 258.9 and 289.8 polyethylene glycol 6000 (Merck, Darmstadt, Germany) in 1 L of distilled water (Michel & Kaufman 1973). Seeds



were incubated in different alternating temperatures 20/10, 25/15 and 35/15 °C day/night in a 12/12-hour light/dark (day/night) photoperiod.

### *Effect of NaCl concentration on seed germination in different temperature regimes*

Solutions of different concentrations of NaCl (Merck, Darmstadt, Germany), including 0 (distilled water), 20, 40, 80 and 160 mM, were prepared (Rao *et al.* 2008). Germination of seeds of the study populations was evaluated in different alternating temperatures including 20/10, 25/15 and 35/15 °C (day/night) in a 12/12-hour light/dark (day/night) photoperiod.

### *Effect of pH on seed germination*

The impact of pH, from 3 up to 11, on germination of seeds of the study populations was evaluated by using the buffer solutions described by Chachalis & Reddy (2000). A 2-mM potassium hydrogen phthalate buffer solution was adjusted to pH 3 or 4 with 1 N HCl. A 2-mM solution of 2-(N-morpholino) ethanesulfonic acid was adjusted to pH 5 or 6 with 1 N NaOH. A 2-mM solution of N-(2-hydroxymethyl) piperazine-N9-(2-ethanesulfonic acid) was adjusted to pH 7 or 8 with 1 N NaOH, and a buffer with a pH of 9 or 10 was prepared with 2-mM N-tris(hydroxymethyl)-methylglycine and adjusted with 1 N NaOH. Seeds were incubated at 25/15 °C (day/night) temperature and a 12/12-hours light/dark (day/night) photoperiod.

### *Statistical analyses*

Experiments were conducted in a complete randomized design with three replicates, with each experiment being performed twice. All data were subjected to arcsin transformation to improve homogeneity. According to Bartlett's test, the transformation of the data of the constant and alternating temperatures, and salt and osmotic stress experiments did not improve homogeneity, and so the analysis was conducted on non-transformed data as percentages. Arcsin transformation improved the pH data and so transformed data were used for analysis.

The data from the alternating and constant temperatures and pH experiments were subjected to analysis of variance (ANOVA) and means were separated by using of Fisher's protected LSD test and standard error (SE).

Regression analysis was performed on the data from the salt and osmotic stress in different temperatures experiment. Germination values (%) for both osmotic potential and NaCl concentration tests were fitted to a functional three-parameter logistic model:

$$G(\%) = G_{\max} / [1 + (x/x_{50})^{G_{\text{rate}}}]$$

where G is the total germination (%) at NaCl concentration x and/or osmotic potential x,  $G_{\max}$  is the maximum germination (%),  $x_{50}$  is the NaCl concentration and/or osmotic potential for 50 % inhibition of the maximum germination, and  $G_{\text{rate}}$  represents the slope.

SigmaPlot, for Windows software, Version 12.0 was used to fit functional three-parameter logistic models.

## Results

### *Alternating temperature and light regimes*

Initial germination of seeds of the *E. caeruleum* study populations differed among the different alternating temperature treatments (Fig. 1). Seed germination under alternating temperatures of population P1 was higher (with the only exception being P2 in 20/10 °C) than those of the other populations both in light/dark and complete darkness conditions. Maximum germination occurred with 25/15 °C (day/night) for P1 (85.19 %). Seed germination in complete darkness was lower than in light/dark conditions for all populations. The initial seed germination of P4 was lower than the other populations in all alternating temperature treatments. Germination percentage was reduced in the highest alternating temperature treatment (35/15 °C) (Fig. 1).

### *Constant temperature and light regime*

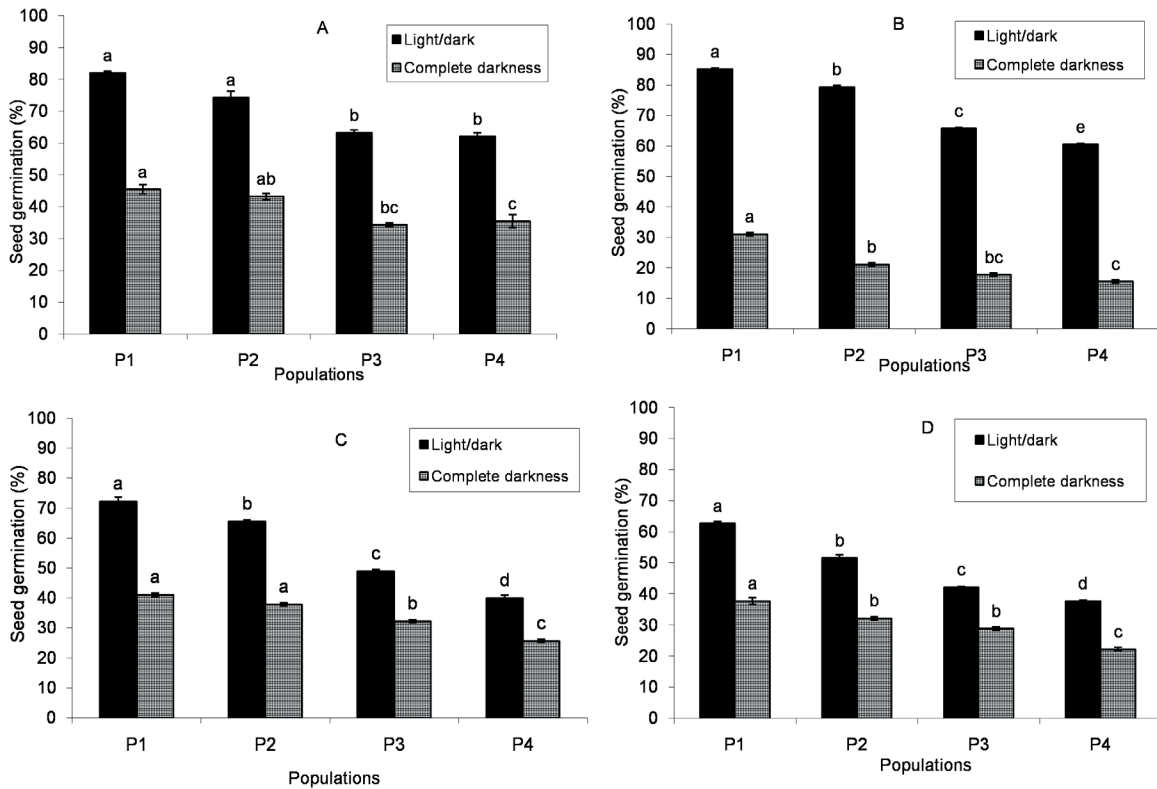
Germination of seeds of the *E. caeruleum* study populations occurred within the constant temperature range of 5 °C to 30 °C in the light/dark regime. Increase in temperature from 5 to 15 °C increased seed germination for all populations in both light/dark and complete darkness photoperiods (Fig. 2). The greatest germination percentage for P1, P3 and P4 was observed at 10 and 15 °C, but seeds of P2 experienced maximum germination in the 15 °C in light/dark regime (Fig. 2B). Germination decreased as temperature increased from 15 up to 30 °C and germination reached zero at 35 °C, both in light/dark and darkness, for all populations (Fig. 2). Both P4 and P3 were exhibited a greater reduction in germination when subjected to temperatures higher than 15 °C in both light regimes. For all populations, seed germination was lower in complete darkness than in the light/dark condition (Fig. 2).

### *Osmotic potential and temperature*

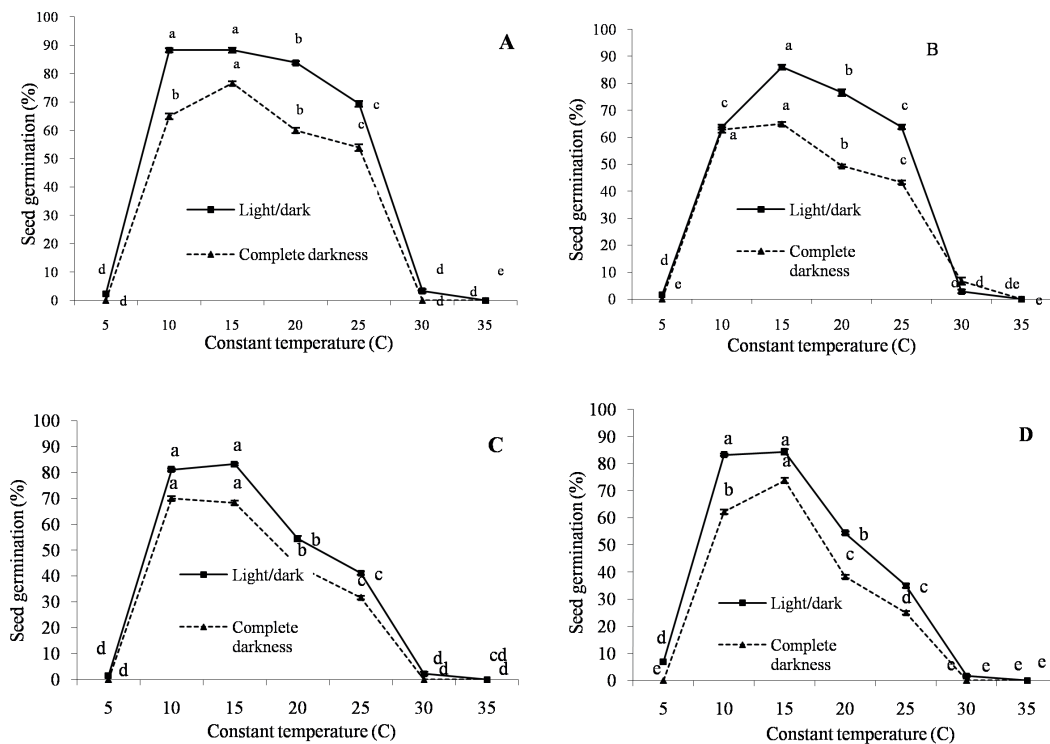
For each population a functional three-parameter logistic model was fitted against the effect of osmotic potential on seed germination in different temperatures. The model predicted the seed germination parameter for populations in different osmotic potentials and temperatures (Fig. 3, Tab. 2). Seed germination of populations reduced as osmotic stress



Effect of some environmental factors on seed germination of *Eryngium caeruleum* M. Bieb. populations



**Figure 1.** Effect of alternating temperatures consist of A=20/10, B=25/15, C=30/10 and D=35/15 °C light/dark on seed germination of *Eryngium caeruleum* populations (P1, P2, P3 and P4) at 12/12 hours light/dark and complete darkness regimes. In each group, different letters at the tops of the standard error bars presents significant differences according to LSD test.



**Figure 2.** Effect of constant temperature on seed germination of *Eryngium caeruleum* populations at 12/12 hours light/dark and complete darkness regimes. A, B, C and D are populations P1, P2, P3 and P4, respectively. In each light regime, different letters at the tops of the standard error bars presents significant differences according to LSD test.

increased. The osmotic stress causing inhibition of 50 % ( $X_{50}$ ) of seed germination increased as alternating temperatures increased (Tab. 2). With increasing temperature to 35/15 °C, the value of  $X_{50}$  increased (Tab. 2).

### NaCl concentration and temperature

A three-parameter sigmoidal model was fitted to the seed germination data of P1, P2 and P3 (Fig. 4). Table 3 shows values of the estimated parameters for populations in the tested temperatures. However, the model could describe well seed germination for P4 under salt stress at different temperatures. Seed germination of P4 reached zero at the salt concentration of 40 mM (Data not shown), which shows that P4 seed germination was more sensitive to salt stress. The maximum germination ( $G_{max}$ ) of all populations decreased with increasing alternating temperatures. The salt stress causing 50 % seed germination of P1 and P2 reduced as alternating temperatures increased (Tab. 3).

Germination in different alternating temperatures and saline stress treatments indicated that germination at lower temperatures was higher than those of higher

temperatures. The populations varied in salt stress tolerance. Seed germination of P2 occurred under a wide range of NaCl concentration from 20 mM up to 160 mM except at temperature 35/15 °C (day/night) (Fig. 4B). Germination of P1 was restricted to a narrower range of salinity, up to 80 mM, for those seeds incubated at temperature 35/15 °C, and also at 40 mM under alternating temperatures 20/10 °C and 25/15 °C (day/night) (Fig. 4A). Seed germination of P3 was restricted to 40 mM salinity at temperatures 25/15 °C and 35/15 °C (day/night) and germination was completely inhibited at higher salinities. At temperature 20/10 °C (day/night), P3 germinated in NaCl concentrations up to 80 mM, with germination reaching zero with an increase in salt stress to 160 mM (Fig. 4C).

### pH solution

Germination of seeds of the *E. caeruleum* study populations differed significantly among pH solutions. For all populations, seed germination occurred in a pH range of 5 to 8. For all populations, the greatest germination observed was in pH 7. Among the populations, P1 had the highest germination rate (Fig 5).

**Table 2.** Parameters estimated for three-parametric logistic equation used in osmotic potential experiment in different temperature.

Populations	Temperature (°C)	$G_{max}$	$x_{50}$	$G_{rate}$	$R^2$	Three-parametric logistic equation
P1	20/10	86.35	0.26	1.95	0.98	$[G (\%) = 86.35 / [1 + (x/0.26)^{1.95}]$
	25/15	81.55	0.29	2.02	0.98	$[G (\%) = 81.55 / [1 + (x/0.29)^{2.02}]$
	35/15	56.47	0.62	27.98	0.99	$[G (\%) = 56.47 / [1 + (x/0.62)^{27.98}]$
P2	20/10	69.80	0.48	6.07	0.97	$[G (\%) = 69.80 / [1 + (x/0.48)^{6.07}]$
	25/15	68.74	0.50	6.03	0.98	$[G (\%) = 68.74 / [1 + (x/0.50)^{6.03}]$
	35/15	52.06	0.52	5.00	0.99	$[G (\%) = 52.06 / [1 + (x/0.52)^{5.00}]$
P3	20/10	66.92	0.48	3.81	0.97	$[G (\%) = 66.92 / [1 + (x/0.48)^{3.81}]$
	25/15	58.48	0.51	4.06	0.97	$[G (\%) = 58.48 / [1 + (x/0.51)^{4.06}]$
	35/15	39.15	0.62	15.79	0.97	$[G (\%) = 39.15 / [1 + (x/0.62)^{15.79}]$
P4	20/10	83.17	0.27	2.05	0.98	$[G (\%) = 83.17 / [1 + (x/0.27)^{2.05}]$
	25/15	56.96	0.40	2.85	0.96	$[G (\%) = 56.96 / [1 + (x/0.40)^{2.85}]$
	35/15	39.72	0.44	3.18	0.98	$[G (\%) = 39.72 / [1 + (x/0.44)^{3.18}]$

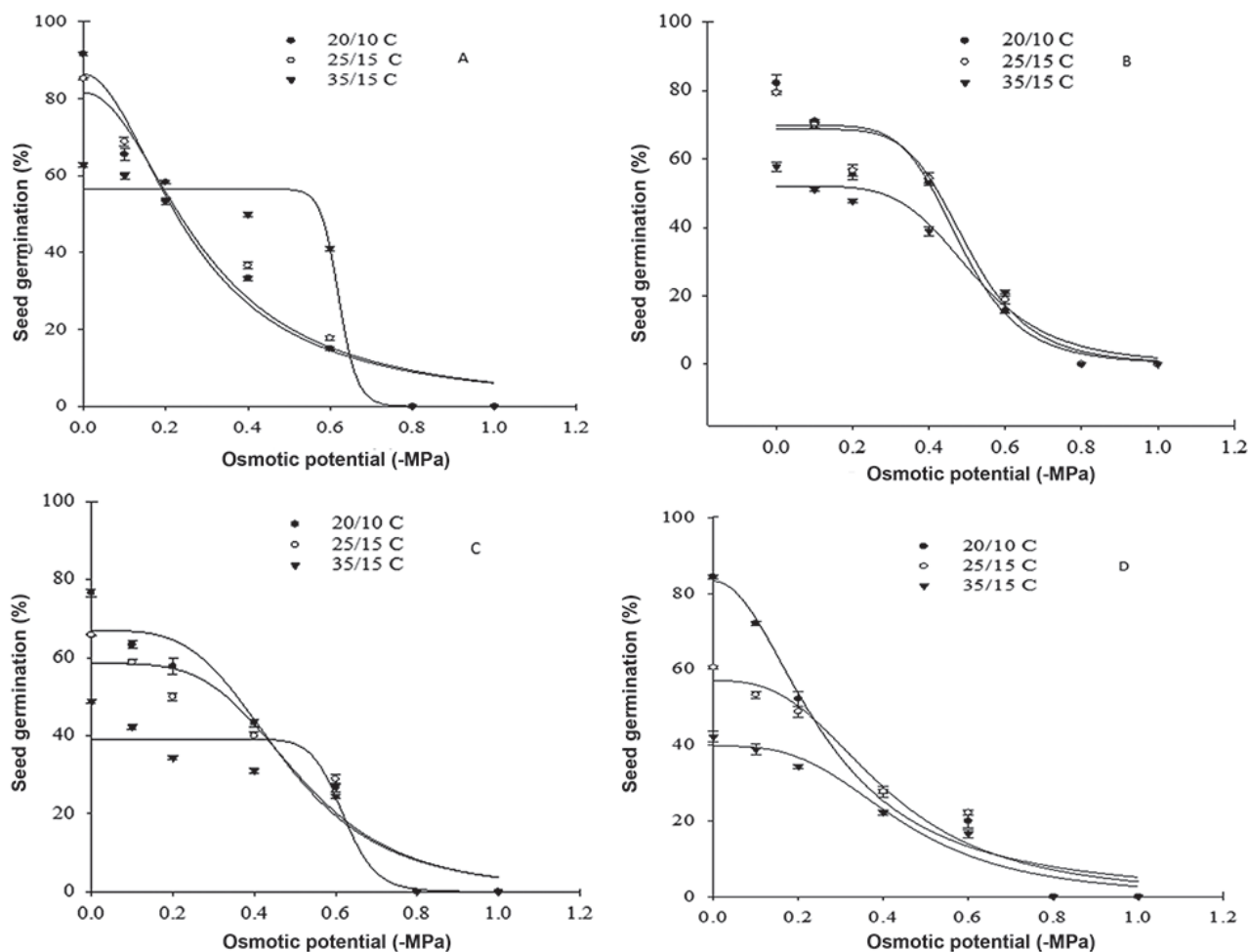
G: total germination (%) in osmotic potential x; Gmax: maximum germination (%); x50: osmotic potential for 50 % maximum seed germination and Grate: curve slope.

**Table 3.** Parameters estimated for three-parametric logistic equation used in salt stress experiment in different temperatures.

Populations	Temperature (°C)	$G_{max}$	$x_{50}$	$G_{rate}$	$R^2$	Three-parametric logistic equation
P1	20/10	86.41	28.21	1.97	0.98	$[G (\%) = 86.41 / [1 + (x/28.21)^{1.97}]$
	25/15	84.83	20.24	1.71	0.98	$[G (\%) = 84.83 / [1 + (x/20.24)^{1.71}]$
	35/15	62.59	17.98	1.31	1	$[G (\%) = 62.59 / [1 + (x/17.98)^{1.31}]$
P2	20/10	75.47	42.30	3.80	1	$[G (\%) = 75.47 / [1 + (x/42.30)^{3.80}]$
	25/15	78.82	33.92	2.23	1	$[G (\%) = 78.82 / [1 + (x/33.92)^{2.23}]$
	35/15	51.25	28.88	2.24	1	$[G (\%) = 51.25 / [1 + (x/28.88)^{2.24}]$
P3	20/10	65.39	20.85	1.87	1	$[G (\%) = 65.39 / [1 + (x/20.85)^{1.87}]$
	25/15	65.72	18.68	2.28	1	$[G (\%) = 65.72 / [1 + (x/18.68)^{2.28}]$
	35/15	42.14	24.99	3.26	1	$[G (\%) = 42.14 / [1 + (x/24.99)^{3.26}]$

G: total germination (%) in NaCl concentration x;  $G_{max}$ : maximum germination (%);  $x_{50}$ : NaCl concentration for 50 % maximum seed germination and  $G_{rate}$ : curve slope.





**Figure 3.** Effect of osmotic potential on seed germination of *Eryngium caeruleum* populations (A=P1; B=P2; C=P3; D=P4) incubated in different temperatures in a 12 hour photoperiod for 15 days. The line represents the functional three-parameter logistic model  $\{G(\%) = G_{max} / [1 + (x/x_{50})^n]\}$  fitted to the data. Vertical bars represents SE.

## Discussion

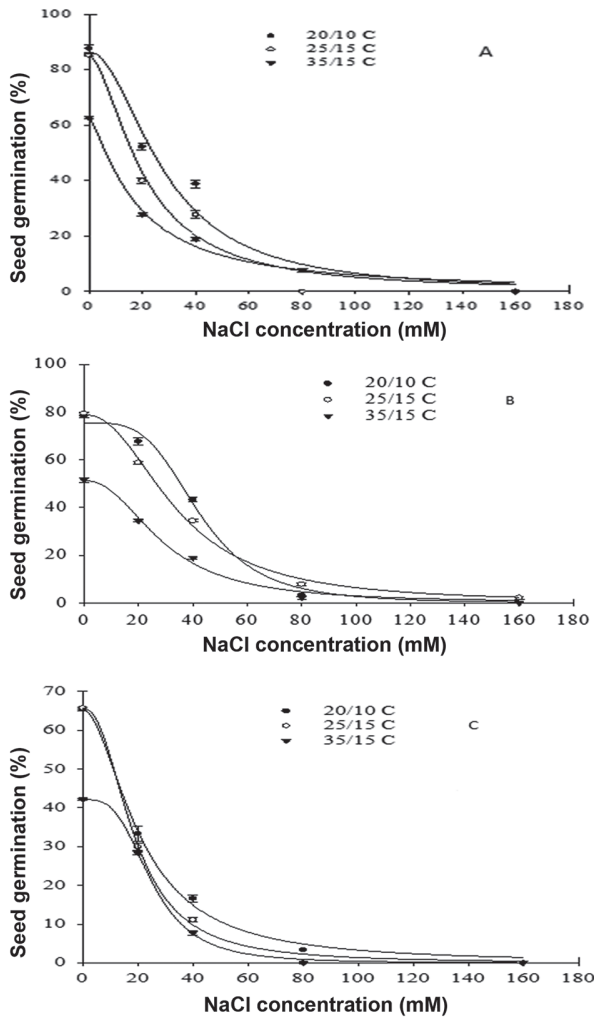
In the present study, the optimum alternating temperature for P1, P2 and P3 was 25/15 °C (day/night). Karimjojeny *et al.* (2014) showed a stimulatory effect of alternating temperature on seed germination of oriental mustard (*Sisymbrium orientale*). Temperature regulates seed germination by affecting enzymatic activity and several other metabolic processes (Chaturvedi *et al.* 2014). Alternating temperature plays an influential role in the balance of plant growth inhibitor and promoter hormones (Copeland & McDonald 2001), where the inhibitor decreases during the low temperature cycle and the promoter increases during the high temperature cycle, leading to germination (Copeland & McDonald 2001).

The optimal temperature for P4 was determined to be 20/10 °C (day/night). Population P4 grows in a region at 1857 m elevation, and with a minimum and average temperature about -15.40 °C and 10.08 °C, respectively (Tab. 1). This population has greater fitness in lower temperatures,

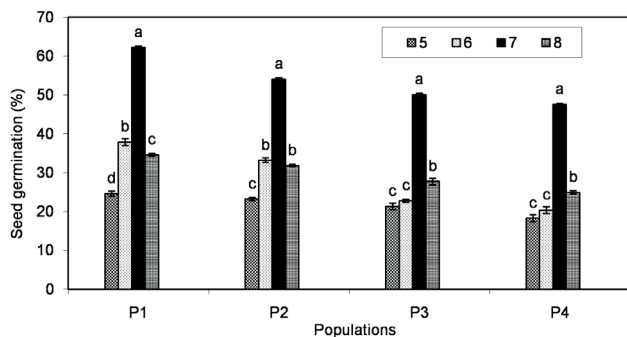
suggesting that the geographical and climatic factors of a region are influential factors in the response of seed germination to temperature.

For all populations, seed germination was lower under complete darkness (35-64 %) than under an alternating photoperiod. The results show darkness is a limiting factor for seed germination of all populations, while photoperiod is an important factor for the enhancement of seed germination. Teuton *et al.* (2004) concluded that in some species seed germination occurs in the presence of light while others may germinate in light or dark regimes. Our findings are in agreement with Opeña *et al.* (2014), who showed that germination of *E. glabrescens* decreased by 52-92 % when exposed to dark. These data suggest that seed germination of *E. caeruleum* will be reduced under plant canopy and litter shade and increasing planting depth.

Temperature is an important factor in determining the timing of germination (Fenner & Thompson 2005). The optimum constant temperature for germination was found to be 10 °C to 15 °C for P1, P3 and P4, and 15 °C for P3. Our data suggest lower (5 °C) and higher (35 °C) temperatures



**Figure 4.** Effect of NaCl concentration on seed germination of *Eryngium caeruleum* populations (A=P1; B=P2; C=P3) incubated in different temperatures in a 12 hour photoperiod for 15 days. The line represents the functional three-parameter logistic model  $\{G (\%) = G_{max} / [1 + (x/x_{50})^n] \}$  fitted to the data. Vertical bars represents SE.



**Figure 5.** Effect of pH solutions on seed germination of *Eryngium caeruleum* population incubated in different temperatures in alternating temperature 25/15 °C day/night and a 12 hour photoperiod for 15 days. In each population, different letters at the tops of the standard error bars present significant differences according to LSD test.

inhibited germination of all populations. A possible reason for this is that low temperatures have inhibitory impacts on catabolic activity, while high temperatures denature proteins and inactivate certain enzymes (Maguire 1973). In the present study, the optimum temperature for germination varied among the different populations. Onen (2006) observed variation in optimum germination temperature among different seed lots of mugwort *Artemisia annua*.

Complete darkness reduced seed germination by up to 53 %, compared to the light/dark regime. Differences in seed germination imposed by light/dark and complete-darkness regimes have been previously reported by Rezvani & Zaefarian (2016) in hoary cress (*Cardaria draba*) and Rezvani *et al.* (2014) in shepherd's purse (*Capsella bursa-pastoris*).

Drought stress during seed germination can delay seedling emergence and reduce seedling survival. Furthermore, osmotic stress can simulate drought stress during germination. Germination of the study populations reduced with increasing osmotic stress at all temperatures. The negative impact of osmotic stress on germination was previously reported by Rezvani & Zaefarian (2016) in hoary cress, and Rezvani *et al.* (2014) in shepherd's purse. Soil water potential is one of the factors that regulate seed water uptake. The rate of seed imbibition decreases with decreasing soil water potential causing a water deficit and, consequently, the decreased germination (Asgarpour *et al.* 2015).

Seed germination in both low levels of alternating temperature and osmotic stress was greater than higher levels. These data suggest that the distribution of *E. caeruleum* is limited to regions with low temperature and drought stress. Also, complete inhibition of germination at osmotic potentials -0.8 MPa, indicate that *E. caeruleum* seeds are sensitive to high osmotic stress in soil, and that germination is restricted to temperate regions with moist soil. In northern Iran, germination of *E. caeruleum* begins in the middle of autumn, and a reduction in rain can potentially lead to a reduction and delay in seedling establishment.

Salt stress reduced seed germination. Seeds often germinate under no-saline conditions, and their germination decreases as salinity increases (Khan & Ungar 1998). Salinity stress influences seed germination by producing an external osmotic potential that prevents water uptake, and also causes toxic ion effects (Hosseini *et al.* 2003). Additionally, sodium ions can alter soil structure and fertility by replacing calcium and magnesium in the anion exchange process, thus leading to nutrient and water stress (Rao *et al.* 2008).

Without considering the temperature, seed germination was inhibited in a range of salinity from 40 mM (for P4) to 160 mM (for P2). Therefore, *E. caeruleum* germination was found to be sensitive to moderately sensitive to saline.

Germination of seeds of the *E. caeruleum* study populations was restricted to a pH range of 5 to 7, with germination in pH 5, 6 and 8 exhibiting a reduction in germination of about 45 %-60% compared to pH 7. Our

data suggests that soil pH may acts as a limiting factor for the distribution of *E. caeruleum*.

Plants vary in their response to pH. Some species require limited range of pH to germinate. Pérez-Fernández *et al.* (2006) showed that pH lower or higher than seven reduced seed germination of *Medicago arabica*, *Epilobium hirsutum*, *Foeniculum vulgare*, *Daucus carota*, *Thapsia villosa*, *Cynosurus cristatus*, *Dactylis glomerata* and *Rumex crispus*. Goubitz *et al.* (2003) concluded that high pH negatively affects seed germination of *Pinus halepensis*. On the other hand, some research has revealed that soil pH is not a limiting factor for many species of plants, such as spotted spurge (*Chamaesyce maculata*) (Asgarpour *et al.* 2015), buffalobur (*Solanum rostratum*) (Wei *et al.* 2009), cadillo (*Urena lobata*) (Wang *et al.* 2009), sheep sorrel (*Rumex acetosella*) (Yazdi *et al.* 2013) and hoary cress (Rezvani & Zaefarian 2016). Consequently, germination over a wide pH range indicates that pH would not be a limiting factor for colonizing different habitats for these species.

## Conclusions

In summary, the optimum alternating temperature for germination of *E. caeruleum* was 25/15 °C in a light/dark regime, and the optimum constant temperature was 10-15 °C, also in a light/dark regime. These data show that successful germination of *E. caeruleum* may be limited to temperate climates. Seed germination of *E. caeruleum* is sensitive to drought stress, while soil with adequate moisture favors germination. Germination percentage decreased and to the point of inhibition at moderate concentrations of NaCl, indicating that salt stress could be a serious obstacle to germination and establishment of *E. caeruleum*. Extreme levels of high and low soil pH also restricted seed germination, and thus soil pH might also be an important limiting factor to this species colonizing new areas. Given the previous lack of data about the conditions for *E. caeruleum* seed germination, the information generated in this study is crucial to understanding the environmental conditions necessary for seed germination and seedling establishment in different habitats. These data will help to predict the potential distribution of *E. caeruleum* in new habitats in the future.

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